

CLAIMS

I claim:

1. A method of coupling a first biomolecule to a second biomolecule, comprising:
(a) covalently attaching a diene moiety to the first biomolecule to form a diene
5 component;

(b) covalently attaching a dienophile to the second biomolecule to form a
dienophile component; and

(c) contacting the diene component with the dienophile component under
conditions that permit a cycloaddition reaction to occur between the components.

10 2. A method of coupling a biomolecule to a gel or solid support, comprising:
(a) covalently attaching a diene moiety to a substrate selected from the group
consisting of the biomolecule and the support, to form a diene component;

(b) covalently attaching a dienophile to the substrate not selected in step (a) to form
a dienophile component; and

15 (c) contacting the diene component with the dienophile component under
conditions that permit a cycloaddition reaction to occur between the components.

3. The method of claim 1, wherein the first biomolecule is a polysaccharide and the
second biomolecule is a polypeptide.

4. The method of claim 3 wherein the polysaccharide is selected from the group
20 consisting of bacterial capsular polysaccharides, fragments thereof, and synthetic analogues
thereof.

5. The method of claim 4, wherein the bacterial capsular polysaccharide is selected
from the group consisting of capsular polysaccharides of *Haemophilus influenzae* type b,
Neisseria meningitidis, Group B *Streptococci*, *Salmonella typhi*, *E. coli*, and *Pneumococci*.

25 6. The method of claim 3 wherein the polypeptide is selected from the group
consisting of bacterial toxins, bacterial toxoids, bacterial outer membrane proteins, keyhole
limpet hemocyanin, horseshoe crab hemocyanin, edestin, mammalian serum albumins,
mammalian gamma-globulins, and IgG-G.

7. The method of any one of claims 1-6 wherein the dienophile moiety is attached to
30 the biomolecule by contacting the biomolecule with 3-sulfosuccinimidyl 4-maleimidobutyrate.

8. The method of any one of claims 3-6 wherein the diene moiety is attached to the polysaccharide by glycosylation of *trans,trans*-hexa-2,4-dien-1-ol with the polysaccharide.

9. The method of claim 7 wherein one of the biomolecules is a polysaccharide, and the diene moiety is attached to the polysaccharide by glycosylation of *trans,trans*-hexa-2,4-dien-

5 1-ol with the polysaccharide.

10. A conjugate of biomolecules prepared by the method of claim 1.

11. A conjugate of a biomolecule with a solid or gel support, prepared by the method of claim 2.

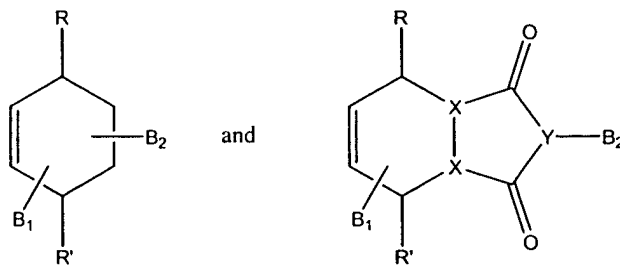
12. A conjugate of biomolecules prepared by the method of any one of claims 3-6.

10 13. A conjugate of biomolecules prepared by the method of claim 7.

14. A conjugate of biomolecules prepared by the method of claim 8.

15. A conjugate of biomolecules prepared by the method of claim 9.

16. A conjugate of biomolecules selected from the group consisting of



15 wherein R and R' are independently H or methyl, or together constitute CH₂, CH₂CH₂, or O; X is CH or N; Y is N, CH=C, or NH-N; and B₁ and B₂ comprise biomolecules independently selected from the group consisting of polypeptides, carbohydrates, polysaccharides, and nucleic acids, and are optionally attached via a linker.

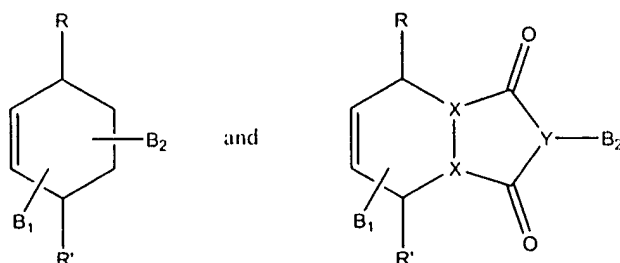
17. The conjugate of biomolecules according to claim 16, wherein one of the
20 biomolecules is a polysaccharide.

18. The conjugate of biomolecules according to claim 17, wherein the polysaccharide is a viral or bacterial polysaccharide.

19. The conjugate of biomolecules according to claim 16, wherein one of the biomolecules is a polysaccharide and the other biomolecule is a polypeptide.

25 20. The conjugate of biomolecules according to claim 19, wherein the polysaccharide is a viral or bacterial polysaccharide.

21. An immobilized biomolecule selected from the group consisting of



wherein R and R' are independently H or methyl, or together constitute CH₂, CH₂CH₂, or O; X is CH or N; Y is N, CH=C, or NH-N; one of B₁ and B₂ comprises a biomolecule selected from the group consisting of polypeptides, carbohydrates, polysaccharides, and nucleic acids and the other of B₁ and B₂ is a solid or gel support, and B₁ and B₂ are optionally attached via a linker.

22. A pharmaceutical composition comprising a conjugate according to any one of claims 10, 11, or 16-20, further comprising a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising a conjugate according to claim 12, further comprising a pharmaceutically acceptable carrier.

24. A pharmaceutical composition comprising a conjugate according to claim 13, further comprising a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising a conjugate according to claim 14, further comprising a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a conjugate according to claim 15, further comprising a pharmaceutically acceptable carrier.

27. A method of inducing, in a mammal, antibodies which immunoreact with a polysaccharide, comprising administering to said mammal a composition according to claim 22, wherein one of the biomolecules is a polysaccharide.

28. A method of inducing, in a mammal, antibodies which immunoreact with a polysaccharide, comprising administering to said mammal a composition according to claim 23.

29. A method of inducing, in a mammal, antibodies which immunoreact with a polysaccharide, comprising administering to said mammal a composition according to claim 24, wherein one of the biomolecules is a polysaccharide.

30. A method of inducing, in a mammal, antibodies which immunoreact with a polysaccharide, comprising administering to said mammal a composition according to claim 25.

31. A method of inducing, in a mammal, antibodies which immunoreact with a polysaccharide, comprising administering to said mammal a composition according to claim 26.

32. An antibody which immunoreacts with a polysaccharide, wherein said antibody is obtained from a mammal, and wherein the production of the antibody by the mammal has been induced by the method of claim 27.

33. An antibody which immunoreacts with a polysaccharide, wherein said antibody is obtained from a mammal, and wherein the production of the antibody by the mammal has been induced by the method of claim 28.

34. An antibody which immunoreacts with a polysaccharide, wherein said antibody is obtained from a mammal, and wherein the production of the antibody by the mammal has been induced by the method of claim 29.

35. An antibody which immunoreacts with a polysaccharide, wherein said antibody is obtained from a mammal, and wherein the production of the antibody by the mammal has been induced by the method of claim 30.

36. An antibody which immunoreacts with a polysaccharide, wherein said antibody is obtained from a mammal, and wherein the production of the antibody by the mammal has been induced by the method of claim 31.

37. An antibody, produced by a hybridoma, which immunoreacts with a polysaccharide, wherein nucleic acid sequences encoding said antibody in said hybridoma are obtained from a mammal in which the production of the antibody has been induced by the method of claim 27.

38. An antibody, produced by a hybridoma, which immunoreacts with a polysaccharide, wherein nucleic acid sequences encoding said antibody in said hybridoma are obtained from a mammal in which the production of the antibody has been induced by the method of claim 28.

39. An antibody, produced by a hybridoma, which immunoreacts with a polysaccharide, wherein nucleic acid sequences encoding said antibody in said hybridoma are obtained from a mammal in which the production of the antibody has been induced by the method of claim 29.

5 40. An antibody, produced by a hybridoma, which immunoreacts with a polysaccharide, wherein nucleic acid sequences encoding said antibody in said hybridoma are obtained from a mammal in which the production of the antibody has been induced by the method of claim 30.

10 41. An antibody, produced by a hybridoma, which immunoreacts with a polysaccharide, wherein nucleic acid sequences encoding said antibody in said hybridoma are obtained from a mammal in which the production of the antibody has been induced by the method of claim 31.

42. A method of inducing passive immunity in a mammal, comprising administering to said mammal an effective amount of an antibody according to claim 32.

15 43. A method of inducing passive immunity in a mammal, comprising administering to said mammal an effective amount of an antibody according to claim 37.

44. A vaccine composition comprising a conjugate according to claim 12, further comprising an adjuvant and a pharmaceutically acceptable carrier.

20 45. A vaccine composition comprising a conjugate according to claim 13, further comprising an adjuvant and a pharmaceutically acceptable carrier.

46. A vaccine composition comprising a conjugate according to claim 14, further comprising an adjuvant and a pharmaceutically acceptable carrier.

47. A vaccine composition comprising a conjugate according to claim 15, further comprising an adjuvant and a pharmaceutically acceptable carrier.